THE HYPOTHERMIC AND ANTI-PYRETIC EFFECT OF PREPARATIONS OF A.C.T.H.

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A common effect of A.C.T.H. in febrile conditions is to lower the body-temperature or even to restore it to normal. This action is usually attributed, by implication at least, to a modification of the disease through the release of adrenal cortical hormones, the lowering of body-temperature being regarded as secondary to this. We find, however, that preparations of A.C.T.H. lower body-temperature even in the absence of pyrexia.

During routine investigation of samples of A.C.T.H., which involved tests for freedom from contamination by pyrogenic material during manufacture, it was observed that all the samples of A.C.T.H. tested lowered the normal body-temperature of the rabbits used.

METHODS

The rectal temperatures of trained docile Himalayan rabbits in good health and weighing about 2 kg. were measured with thermocouples enclosed in rubber catheters and inserted 6 in. into the rectum. The animals sat in metal boxes, being restrained only by a metal collar round their necks. Under these conditions, they remained quiet for many hours and retained the catheters in situ during the whole period of the test. The room-temperature was maintained between 19° and 22°C. Injections were made into the marginal vein of the ear with pyrogen-free glassware, needles, and saline solution.

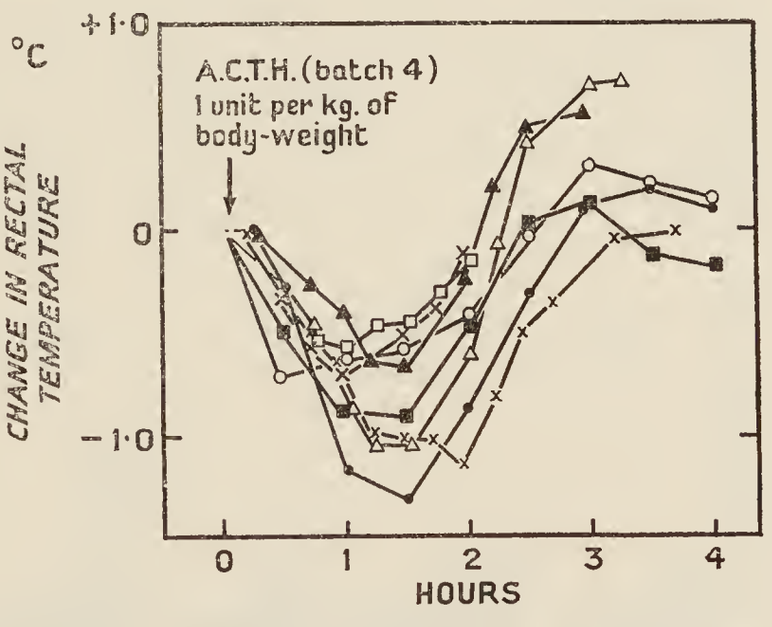

Fig. 1—Effect of intravenous A.C.T.H., batch 4, 1 unit per kg. of body-weight on body-temperature of eight rabbits.

Various samples of A.C.T.H. were used, each of which had been submitted at various times to be tested for freedom from pyrogens. We have given them arbitrary batch numbers 1–9. We shall usually refer to this material simply as A.C.T.H., although it certainly contains other substances.

For experiments with posterior pituitary hormone, extracts were made as described by Burn (1937) from samples of the Pituitary (Posterior Lobe) International Standard.

To induce artificial pyrexia, a preparation of dried bacterial bodies from a strain of *Proteus vulgaris* was used, for which we are grateful to Dr. A. A. Miles. This material has been shown previously (F. C. MacIntosh and W. D. M. Paton, unpublished) to raise the body-temperature 1° or 2°C in these rabbits, when injected in a dose of 1 μg. per kg. of body-weight ; this pyrexial response typically begins in about 20–30 min., lasts for over 5 hours, and displays a transient partial defervescence 2–3 hours after the injection.

RESULTS

Hypothermic Effect of A.C.T.H. Preparations

Each of the various batches of A.C.T.H. lowered the rectal temperature when injected intravenously. The fall in temperature began within 15 minutes of injection and progressed steadily to reach its lowest point in about $1^1/_2$ hours. Recovery then took place and followed a somewhat similar time course, normal temperature being restored about 3 hours after the injection. Fig. 1 shows a typical range of responses obtained by the injection of a single sample (batch 4) in a dose of 1 unit per kg. of body-weight into each of eight rabbits. The response varies with the sample of A.C.T.H., with the dose given, and with the animal used, and considerably larger falls in temperature were sometimes observed. Thus, out of twenty-five tests with various batches at this dose level (1 unit per kg. of body-weight), the temperature fell more than 1°C on twelve occasions ; with a larger dose, 3 units per kg. of body-weight, the temperature fell more than 2·5°C in four out of twenty-one tests.

The values obtained in our experiments are shown in the accompanying table. Many of the tests were made on rabbits which had already been injected with A.C.T.H. on one or more occasions ; since it was possible that previous exposures to A.C.T.H. might affect the response, the number of the exposure is given in parentheses after each result. Two main points emerge from consideration of

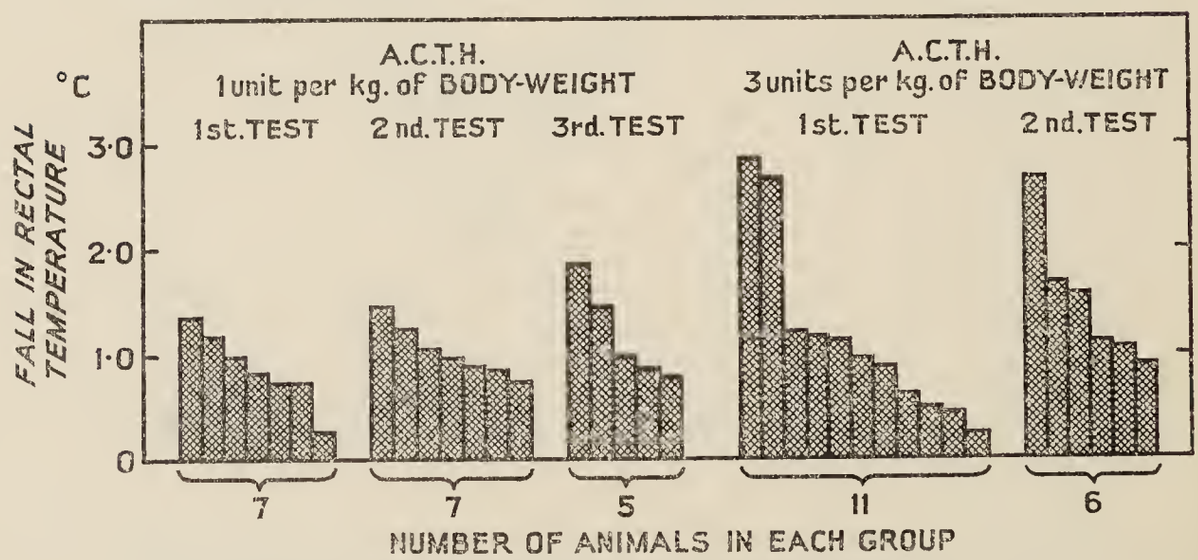

Fig. 2—Hypothermic responses of rabbits to A.C.T.H. 1 unit or 3 units per kg. of body-weight, with repeated tests on the same animals.

REDUCTIONS IN BODY-TEMPERATURE (°C) PRODUCED BY INTRAVENOUS A.C.T.H.

Rabbit no.	A.C.T.H. batch no.									Dose (units per kg. of body-weight)
	1	2	3	4	5	6	7	8	9	
12	..	..	1·17 (*1*)	..	..	..	0·8 (*3*)	..	..	
13	..	..	1·84 (*3*)	..	..	1·25 (*2*)	1·97 (*5*)	..	..	
15	..	..	1·01 (*4*)	..	..	1·45 (*3*)	1·04 (*6*)	..	..	
16	..	..	..	0·5 (*4*)	..	0·99 (*3*)	..	..	..	
24	..	..	..	..	..	..	..	..	1·52 (*4*)	
									0·84 (*3*)	
25	..	..	..	..	..	..	..	..	0·98 (*3*)	1
37	..	..	..	0·74 (*1*)	..	..	..	1·45 (*2*)	..	
38	..	..	..	1·34 (*1*)	..	..	..	0·84 (*2*)	..	
39	..	..	..	0·97 (*1*)	..	..	..	..	..	
40	..	..	..	0·87 (*2*)	0·24 (*1*)	..	..	..	..	
41	..	..	..	0·71 (*2*)	0·74 (*1*)	..	..	..	..	
42	..	..	..	1·25 (*3*)	0·82 (*1*)	..	..	1·05 (*2*)	..	
3	..	..	1·19 (*1*)	..	2·66 (*2*)	..	..	..	..	
8	..	0·93 (*1*)	..	..	1·49 (*2*)	..	..	..	..	
12	..	..	..	..	..	..	..	..	1·07 (*2*)	
13	..	1·16 (*1*)	..	..	..	..	..	..	1·66 (*4*)	
15	0·48 (*1*)	0·88 (2)	..	..	..	..	..	..	0·74 (*5*)	
16	..	..	1·13 (*1*)	..	..	..	1·32(*3*)	..	..	
17	..	..	0·59 (*1*)	..	..	..	..	..	..	
18	0·45 (*1*)	..	..	..	1·64 (*2*)	..	..	..	..	3
22	..	..	..	0·89 (*1*)	..	..	..	..	..	
24	..	..	..	2·67 (*1*)	..	..	..	..	..	
				2·76 (*4*)						
25	..	..	..	2·83 (*1*)	..	..	..	..	..	
				1·65 (*4*)						
26	0·21 (*1*)	..	..	..	..	..	..	..	..	

Numerals in parentheses after each result give number of exposure to A.C.T.H. for animal concerned.

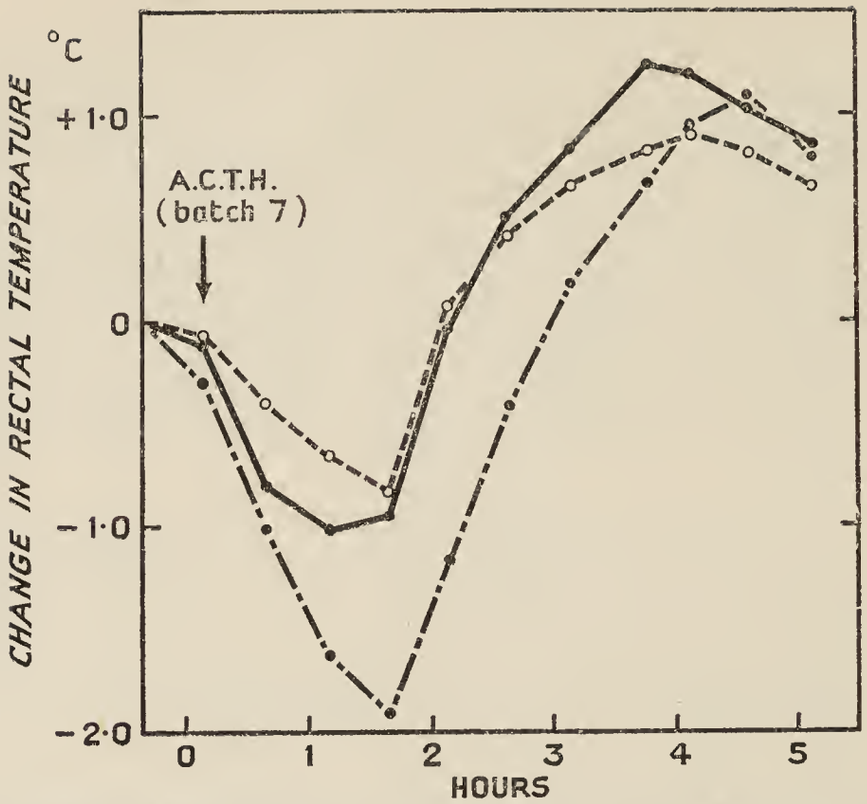

Fig. 3—Effect of intravenous A.C.T.H., batch 7, 1 unit per kg. of body-weight on body-temperature of three rabbits.

the data in this table : (1) with the doses studied (1 and 3 units per kg. of body-weight), each batch of A.C.T.H. caused a fall in body-temperature on every occasion it was tested ; and (2) this hypothermic action was observed not only with the first dose of A.C.T.H. in any rabbit but also with all subsequent injections in the same rabbit. From the data it is also clear that the hypothermic response to a given dose of A.C.T.H. may vary in different animals, or even in the same animal, from test to test. The response, moreover, appeared to be greater with bigger doses and tended to increase slightly on a second or third test (fig. 2).

Preliminary experiments have shown that this hypothermic action of A.C.T.H. also follows subcutaneous and intramuscular administration, and that intravenous doses as small as 0·1 unit per kg. of body-weight may be active.

Antipyretic Action of A.C.T.H. Preparations

It has been mentioned that these batches of A.C.T.H. had been submitted to scrutiny for possible contamination with pyrogenic substances. Normally these pyrogens are detected by the appearance of pyrexia, and the hypothermic action we observed must obviously interfere with such a test. Batch 4 appeared to be particularly free from such activity, but in some samples the hypothermic effect was succeeded by a considerable rise in temperature (fig. 3). Since such a rise did not take place in all the batches tested, whereas the hypothermic effect did, the subsequent rise in temperature could not be a consequence of the hypothermia—e.g., a sort of "rebound" phenomenon. The character and time course of the rise in temperature, when it occurred, was compatible with that of pyrogenic contamination. If

this reasoning were correct, it followed that A.C.T.H. was modifying the pyrogenic response; for normally the pyrexia from intravenous injection of common pyrogenic materials is well established within an hour of injection and near its highest point about another hour later, whereas in these tests (as in fig. 3) the onset of pyrexial response, if any, appeared much later, being delayed by $1^1/_2$–2 hours (a time corresponding approximately to the hypothermic phase of the A.C.T.H.).

Our experiments showed that A.C.T.H. interferes with pyrogenic activity in the way suggested. In the experiments recorded in fig. 4 the response to proteus pyrogen (1 μg. per kg. of body-weight) in three rabbits was compared with the response to this pyrogen injected with A.C.T.H. 1 unit per kg. of body-weight (batch 4) in another three rabbits. The rabbits treated with pyrogen alone showed the typical brisk rise in temperature, whereas those which received A.C.T.H. in addition gave a more sluggish response, the pyrexia being delayed, reduced, and preceded by a fall in temperature. In fact, the effect of injecting pyrogen with A.C.T.H. was akin to the response obtained with the more pyrogenic batches of A.C.T.H. (fig. 3). Our conclusion that these samples were contaminated with pyrogen was thus corroborated.

It was shown that A.C.T.H. could not only delay the onset of a pyrogenic response but also reduce an established pyrexia. Pyrexia was established in two rabbits by injection of the pyrogen 1 μg. per kg. of body-weight; at the height of the pyrexia one rabbit was given intravenous A.C.T.H. 1 unit per kg. of body-weight, and the temperature rapidly fell to normal; but in the rabbit

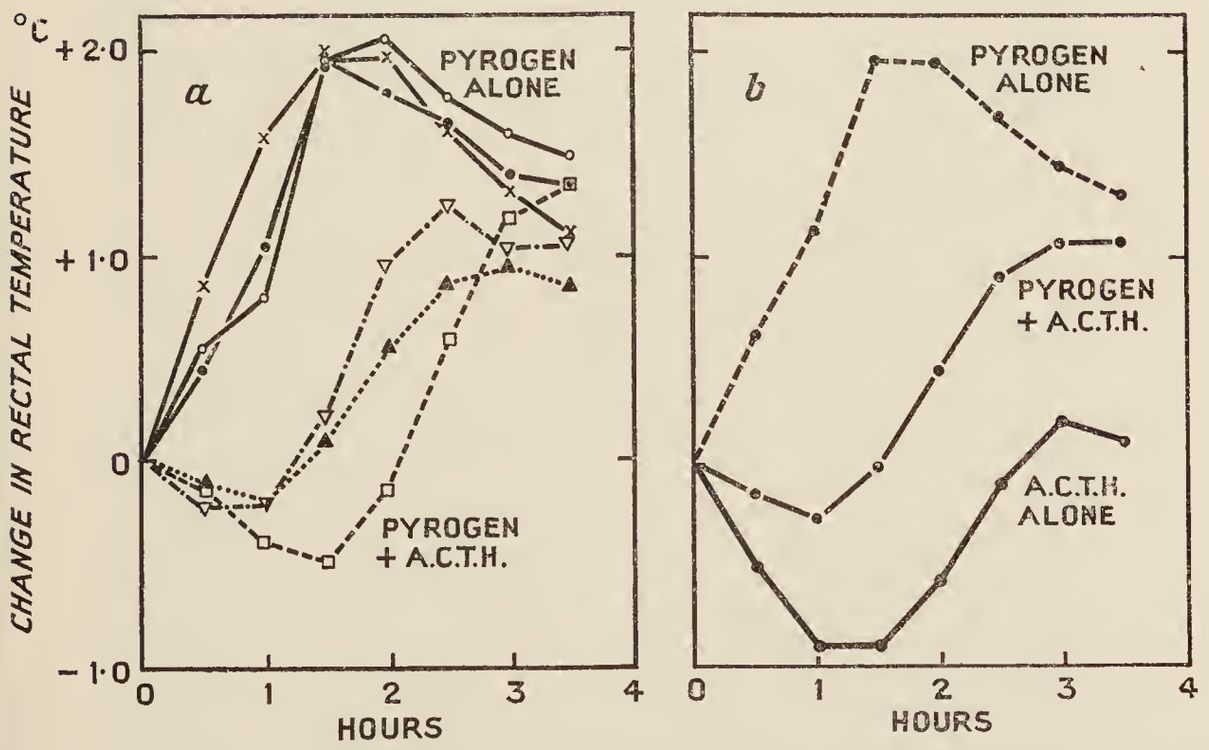

Fig. 4—*a*, upper curves, pyrexial responses to intravenous proteus pyrogen 1 μg. per kg. of body-weight in 3 rabbits; lower curves, responses to intravenous proteus pyrogen 1 μg. per kg. of body-weight and A.C.T.H., batch 4, 1 unit per kg. of body weight in 3 rabbits; *b*, uppermost and middle curves, data of *a* averaged; lowermost curve, average of responses to A.C.T.H., batch 4, 1 unit per kg. of body-weight alone in 3 rabbits.

not treated with A.C.T.H. the pyrexia persisted. The experiment was repeated three days later on the same animals with treatment reversed, and the averaged results are presented in fig. 5.

Effects of A.C.T.H. on Body-temperature not due to Content of Posterior Pituitary Hormone

The material used in these experiments—i.e., commercial preparations of purified A.C.T.H. for clinical use—is not completely pure; hence the effects on body-temperature cannot with certainty be attributed to this hormone. The consistency of these effects suggests that, if they are not due to A.C.T.H., they must be caused by some other material associated with A.C.T.H. from the earliest stages of preparation, rather than to some casual contamination (comparable to that which renders only some of the preparations pyrogenic). One such material is the hormone of the posterior lobe of the pituitary gland, which was known to be present in small amounts in every batch. The stated activity ranged from 0·5 to 1·5 units of oxytocic activity per 100 units of A.C.T.H.

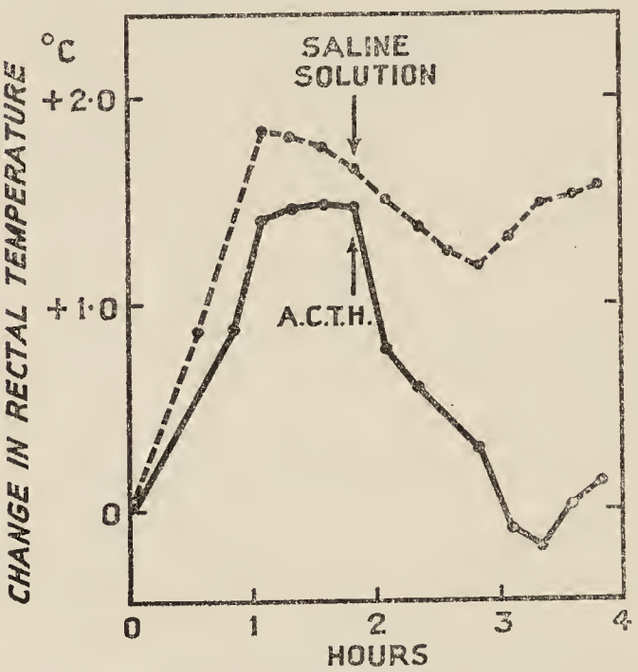

Fig. 5—Pyrexial responses to proteus pyrogen 1 μg. per kg. of body-weight, with and without the injection of A.C.T.H. 2 hours after injection of pyrogen (cross-over test, 2 rabbits, results averaged).

We have therefore compared the effects of A.C.T.H. on the body-temperature with those of a posterior-pituitary preparation. In fig. 6 are shown the results of an experiment in which, in two animals, posterior-pituitary extract 0·015 unit per kg. of body-weight was first injected intravenously, without significant effect on the body-temperature; 3 hours later, A.C.T.H. (batch 4) 1 unit per kg. of body-weight was injected, giving the usual vigorous hypothermic response. The results of another experiment, using a cross-over method on two animals, and a higher dose of posterior pituitary (0·1 unit per kg. of body-weight) are shown in fig. 7. Even this large dose of posterior-pituitary extract did not lower the body-temperature, although both animals responded to A.C.T.H. in the usual way.

Just as the presence of small amounts of posterior-pituitary hormone cannot explain the hypothermic actions of the A.C.T.H. preparations, so does it fail to

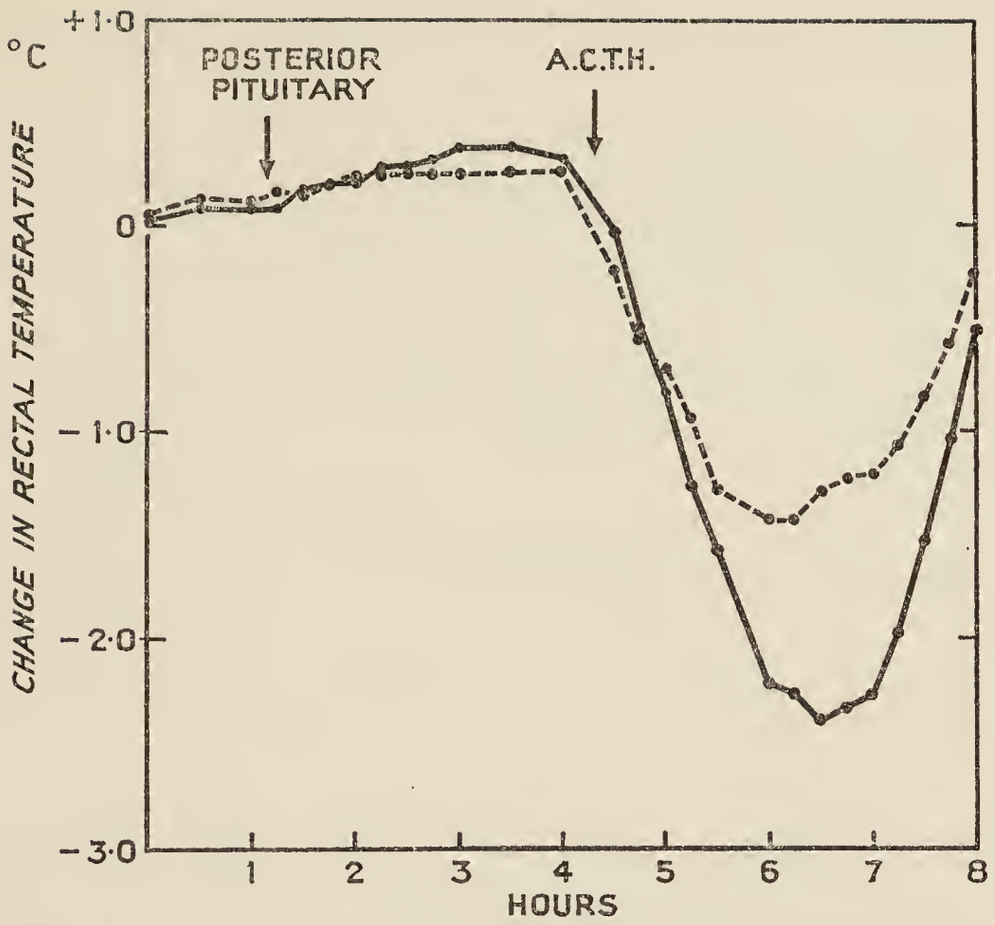

Fig. 6—Responses in 2 normal rabbits : *a*, to posterior-pituitary extract 0·015 unit per kg. of body-weight ; *b*, 3 hours later, A.C.T.H., batch 4, 1 unit per kg. of body-weight.

explain the antipyretic effects. In fig. 8 is shown a comparison in a cross-over test on two animals between the effects of A.C.T.H. 1 unit per kg. of body-weight and of posterior-pituitary hormone 0·1 unit per kg. of body-weight on the pyrexia produced by the proteus pyrogen. The course of the pyrexia is hardly influenced at all by the posterior-pituitary hormone, whereas A.C.T.H. in the same animals produced its usual effect.

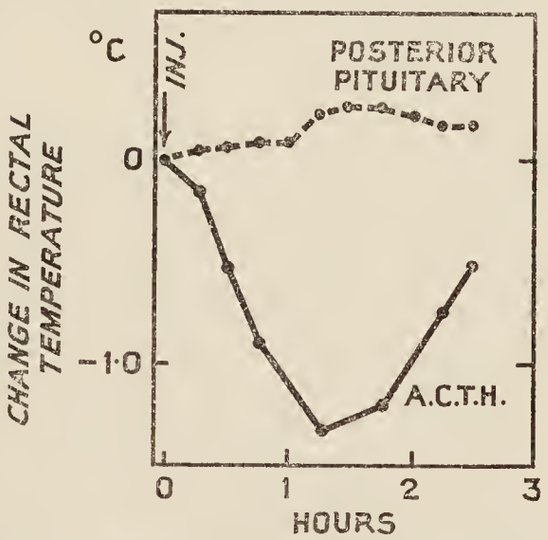

Fig. 7—Responses in 2 normal rabbits to posterior-pituitary extract 0·1 unit per kg. of body-weight and to A.C.T.H., batch 4, 1 unit per kg. of body-weight (cross-over test, results averaged).

DISCUSSION

Our experiments have shown that preparations of A.C.T.H. will lower the body-temperature of normal rabbits, will delay and reduce the pyrexial response of rabbits to a dose of pyrogen, and will reduce an established pyrexia. This action is not due to contamination with posterior-pituitary hormone. An antipyretic action of A.C.T.H. has already been reported (Kass and Finland 1950), but the hypothermic action does not seem

to have been recorded. Cortisone, given for several days previously, is known to reduce the sensitivity of rabbits to a pyrogen (Recant et al. 1950).

The importance of these observations for the testing for freedom from pyrogens is obvious. The usual pyrogen test (British Pharmacopœia 1948) ends 3 hours after the test material has been injected. The ability of A.C.T.H. preparations to delay the appearance of a pyrogenic response means that a longer test period, preferably 5 hours, must be used. The difficulties of interpreting the results of such a test, in which what is essentially a mixture of drugs of opposing actions is injected, need no emphasis; this problem can hardly be discussed, however, in the absence of decisive information about the sensitivity of man to such mixtures and about the dose-response characteristics of these substances in man.

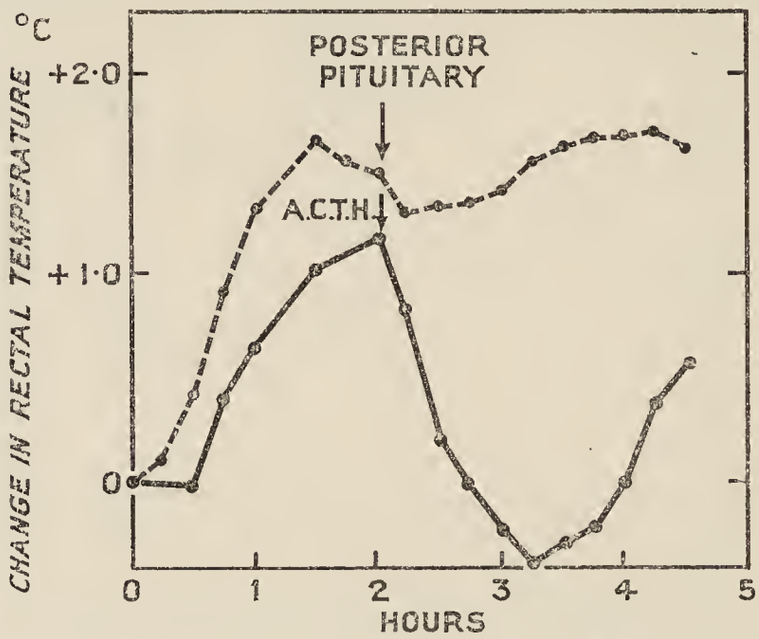

Fig. 8—Responses in 2 rabbits treated with proteus pyrogen 1 µg. per kg. of body-weight to: *a*, posterior-pituitary extract 0·015 unit per kg. of body-weight; and *b*, A.C.T.H. 1 unit per kg. of body-weight (cross-over test, results averaged).

But the existence of this hypothermic and antipyrexial action of A.C.T.H. preparations has considerably wider significance than for the control of purity of a drug. It suggests that the pituitary may, under some conditions, play some part in the normal physiology of temperature regulation. In addition, the effects on body-temperature are displayed by doses comparable to those used clinically in man, and the possibility arises that the extensive metabolic or vascular readjustments involved in bringing about such considerable falls in temperature, or the fall in temperature itself, may be of therapeutic importance. The effects of A.C.T.H. preparations in a given disease might in part be due, therefore, not to a specific action on the disease but rather indirectly to such vascular or metabolic changes.

SUMMARY

Preparations of A.C.T.H. cause a fall in the rectal temperature of normal (unanæsthetised) rabbits. The fall in temperature is about 1°C (range 0·24°–1·97°C)

with a dose of 1 unit per kg. of body-weight, reaches its lowest point in 1–2 hours, and passes off in about 3 hours.

A.C.T.H. preparations, given simultaneously with a pyrogen, will delay and reduce the pyrexial response to the pyrogen. Given after pyrexia has been established, A.C.T.H. preparations will temporarily lower the temperature to normal or below.

The hypothermic and antipyretic actions of A.C.T.H. preparations were displayed by all samples tested, and were not due to contamination with posterior-pituitary hormone.

The significance of these actions in routine pyrogen testing of A.C.T.H. preparations and in relation to physiological processes involved in temperature regulation is discussed.

We are deeply indebted to Mr. C. Pergande for his help in these experiments.

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